Characterization of 3-aminobiphenyl and 4-aminobiphenyl hemoglobin adducts in adult smokers phenotyped for CYP1A2 and NAT2 activity

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INTRODUCTION

- Human ex[illegible]ure to aromatic amines can occur from various sources including [illegible], environmental exposure and tobacco smoke inhalation [1-5].
- Aminobiphenyl (ABP) is one of the aromatic amines that arises from cigarette smoke. Of the various ABP's, 4-ABP is classified by IARC as a bladder carcinogen and human exposure occurs from cigarette smoke as well as other sources; 3-ABP on the other hand is not a carcinogen and is found mainly in cigarette smoke.
- Hepatic N-hydroxylation by the cytochrome P450 enzyme, CYP1A2, is the critical step in the metabolic activation of 4-ABP[6, 7]. Conjugation by N-acetyltransferase (NAT) and glucuronidation competes with N-hydroxylation leading to detoxification [8,9]. The N-hydroxylamine in blood is heme-mediated oxidized to the nitrosoarene, which reacts with the sulfhydryl group of cysteine in hemoglobin (Hb), resulting in a stable Hb adduct.
- The biologically effective dose of the ultimate carcinogen depends on the rate of formation of the N-hydroxylamine, which is mainly influenced by the balance between N-acetylation and N-hydroxylation of the parent amine. Due to the presence of allelic variants of NAT1 and NAT2 and the induction of CYP1A2 in smokers, this balance can be perturbed in some individuals. Studies have show[illegible] correlation between Hb adduct formation and DNA adduct levels, sug[illegible]g that ABP Hb adducts could provide a measure of the biologically effective dose.

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1) To [illegible] the [illegible] CYP1A2 and NAT2 phenotype on 3-ABP and 4-ABP he[illegible] (Hb) a[illegible] levels
2) To [illegible] the intra- and inter-i[illegible] [illegible]y of 3-ABP and 4-ABP Hb adduct levels.

METHODS

Study [illegible]

This was [illegible] single center, non-confined, parallel group study design. The study was conducted at Covance Clinical Laboratories, Madison, Wisconsin under a protocol approved by the IRB. The subject population consisted of 136 healthy adults of 21 years or older [smokers (n=68, 30 males and 38 females) and non-smokers (n=68, 32 males and 36 females)].

Sample Collection

Blood samples were obtained from smokers and nonsmokers for Hb adduct determinations on two different occasions separated by 5 weeks. Urine samples were collected for caffeine phenotyping at week 1. Subjects refrained from consuming beverages containing caffeine or alcoholic drinks for a minimum of 8 hrs before and during the day of urine collection. After emptying their urinary bladder, subjects ingested 200 mg of caffeine (No-Doz®, 2 tablets at 100 mg each) with 250 ml of water. Consumption of liquids was not restricted during the study. Urine was collected after 4-5 hrs. A 10 ml aliquot of the collected urine (after recording the original volume) was adjusted to pH 3.5 by addition of 200 mg of ascorbic acid, immediately frozen, and stored at –20 °C until analysis.

CYP1A2 and NAT2 Phenotyping

Caffeine and its metabolites were quantified according to the procedure described by Butler *et al.* (10) with some minor modifications. Caffeine metabolite ratios were used for phenotyping as follows:

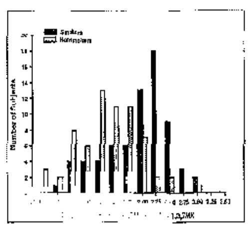
$$\text{CYP1A2 activity} = \frac{\text{(1,7-Methyluric acid + 1,7-Methylxanthine)}}{\text{1,3,7-Trimethylxanthine (Caffeine)}}$$

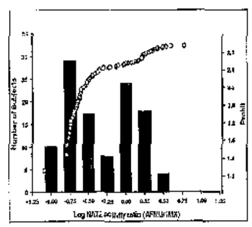
$$\text{NAT2 activity} = \frac{\text{AFMU (5-acetylamino-6-formylamino-3-methyluracil)}}{\text{1-Methylxanthine}}$$

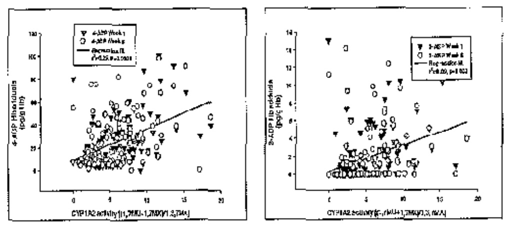
Hemoglobin Adduct Measurements

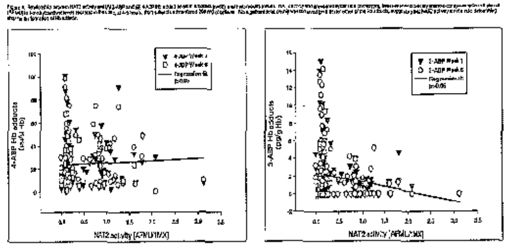
The hemoglobin adducts of 3-aminobiphenyl and 4-aminobiphenyl were measured at Covance Laboratories Limited, Harrogate, England. Briefly, the RBC samples were lysed, centrifuged and supernatant dialyzed over a period of 2-3 days. The concentration of hemoglobin was determined before hydrolysis of the 3-ABP and 4-ABP hemoglobin adducts. Liberated ABPs were then concentrated using solid-phase extraction (C_{18} reversed phase), eluted with chloroform. After evaporating to dryness, the ABPs were then derivatized with pentafluoropropionic anhydride in hexane, taken to dryness using a vacuum centrifuge and reconstituted in ethyl acetate prior to analysis using capillary GC-MS. The lower limit of quantification (LLOQ) of the method was 0.5 pg/g Hb.

RESULTS

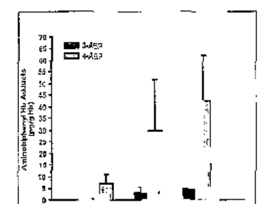








	Smokers			Non-Smokers		
Hb Adducts	Gender			Gender		
	Female	Male	Both	Female	Male	Both
3-ABP						
Week 1 - N	38	30	68	36	32	68
Mean±SD	3.16±2.53	3.97±4	3.52±3.26	0.28±0.8	0.08±0.2	0.18±0.6
Range	0 - 10.4	0 - 15.0	0 - 15.0	0 - 4.59	0 - 0.89	0 - 4.59
Week 6 - N	38	30	68	36	32	68
Mean	3.60±3.57	3.91±3.67	0.7±3.0	0.15±0.43	0.12±0.49	0.13±0.45
Range	[illegible]	0 - 12.7	[illegible]	[illegible]	0 - 2.5	0 - 2.5
4-ABP						
Week 1 - N	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Mean	27.30±13.80	42.27±25	34.1±20.7	8.1±4	7.64±5.01	7.64±4.9
Range	0 - 67.80	13.4 - 100.6	0 - 100.6	2.6 - 23.2	2.9 - 20.7	2.6 - 20.7
Week 6 - N	38	30	68	36	32	68
Mean	38.65±20.61	38.6±21.7	38.6±20.9	8.6±3.86	9.32±7.9	9.02±6.1
Range	0 - 90.62	0.6 - 87.8	0 - 87.8	4.1 - 22.7	0 - 40.6	0 - 40.6



[illegible]

- Results from this study corroborate previous observations.
- The levels of 3-ABP and 4-ABP Hb adducts were significantly higher in smokers compared to nonsmokers
- Significant correlations were observed between number of cigarettes smoked with the ABP Hb adduct levels indicating cigarette smoke is a major source for ABP in smokers
- Detectable levels of ABP Hb adducts in nonsmokers suggest that ABP exposure occurs from other sources e.g., dietary, environmental.
- For 53% of the nonsmokers and 6% of the smokers, the 3-ABP Hb adducts were below detectable levels
- Measurable levels of 4-ABP adducts were observed in both groups
- 3-ABP Hb adduct levels were about 10-fold lower than 4-ABP Hb adducts in the smokers
- 4-ABP Hb adduct levels were higher in male smokers compared to female smokers
- CYP1A2 activity was better correlated with the ABP Hb adducts than NAT2, suggesting that CYP1A2 but not NAT2 plays an important role in ABP metabolism
- The inter-individual variability was greater for 3-ABP (~80% coefficient of variation, CV) compared to 4-ABP (~52% CV)
- Although there appears to be less confounding factors in the measurement of 3-ABP Hb adducts in nonsmokers, its utility as a biomarker of exposure to cigarette smoke is limited due to the low levels observed in smokers

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2) T[illegible] [illegible] of fumes from heated [illegible]
3) T. Pl[illegible] [illegible]genic [illegible] [illegible]ines from [illegible]
4) S.R. Myers, M.T. [illegible], T. Pl[illegible] [illegible] adducts in maternal and fetal blood. Poly[illegible] [illegible]omatic Com[illegible]
5) J.W. Cullinga, [illegible] [illegible]biphenyl hemoglobin adducts and arom[illegible] [illegible]A adduct[illegible] [illegible]mokers as bi[illegible] [illegible]ers of exposure. *Cancer Epidemiology, Biomarkers and Preven[illegible]* [illegible]).
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